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Original Article

Effects of Varying Essential Oil Mixture Concentrations Applied Underconditions of Different Temperature Stress on Cardiac Markers and Other Blood Parameters

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■Keywords

Broiler, Cardiac troponin I (cTnI), Erythrocyte distribution width (RDW), Essential fatty acid mixture, Temperature stress.

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ABSTRACT

Cardiac troponin (cTnI) and erythrocyte distribution width (RDW) have been used as specific markers for the evaluation of cardiac arrhythmias and myocardial diseases. In this experimental study, we aimed to determine the dose-dependent administration of an Essential OilMixture (EOM) *(Eucalyptus globulusLabill, Thymus vulgaris, Cymbopogon nardus, and Syzygiumaromaticum)* on the serum erythrocyte indices and myocardial damage. We used 400 male, one-day-old, Ross-308 chicks. Each group ((n=8) (22°C Control (C), C+250 mL/1,000 L, C+500 mL/1,000 L, C+750 mL/1,000 L), 36°C (stress control (SC), SC+250 mL/1,000 L, SC+500 mL/1,000 L, SC+750 mL/1,000 L) was further divided into eight groups with 50 animals. Each group of 50 chicks was further subdivided into five groups with 10 animals in each compartment. The results showed that the WBC, RBC, HGB, HCT, RDW-SD, RDW-CV, and Lymphocyte decreased in groups without exposure to temperature stress (22°C) compared to the 22°C control group; whereas, CnTnI, MCHC, NEUT, and IG (p<0.05) increased compared to the control group. In groups exposed to temperature stress (36°C), the WBC, HGB, HCT, RDW-SD, and Lymphocyte decreased compared to the control group, but MCH, MCHC, NEUT, and IG increased compared to the control. However, cTnI, CK, creatinine, RBC, and RDW had no effect on CD, MONO, EO, and BASO (p<0.05). EOM mixture had no effect on hematological and biochemical parameters.

INTRODUCTION

In recent years, there have been various global developments ın order to improve product quantity and quality in line with the wishes of Consumers to meet the enhanced nutritional needs of the rapidly growing world population. However, fattening performance is significantly affected by heat stress in broiler hens owing to global warming. Broilers have a higher basal metabolic rate, as they have a high rate of feed utilization as a genetic characteristic (Mohamed *et al.*, 2012; Mahmoud *et al.*, 2015). Given the rapid development of and stress exposure in broilers and the sudden death syndrome associated with cardiovascular diseases due to lubrication in internal organs, broilers breeding facilities are associated with high mortality. Studies have shown that the mortality rate is doubled in birds fed high-protein diets (Breeding *et al.*, 1994; England *et al.*, 2017). Cardiomyopathies are diseases of the heart muscle that result from heart failure and cause cardiac dysfunction. The disease has a high morbidity and mortality rate and results in sudden death. Cardiomyopathies are associated with decreased diastolic volume especially in the ventricles, a dilated atrium, limited ventricular filling and rarely, ventricular myocardial fibrosis. In this case, fat accumulation is caused by the prevention of electrical

conduction and arrhythmias (Maron *et al.*, 2006; Wieczorek *et al.*, 2008; Wexler *et al.*, 2009; England *et al.*, 2017).

Cardiac troponin (cTnI) is an important protein that contributes to the regulation of actin-myosin, which is an essential component of striated-muscle contraction. Troponin has three basic globular (TnI, TnT, and TnC) proteins on striated muscles. These proteins contribute to tropomyosin-troponin binding (troponin-T), actin-myosin contraction by regulating actin and myosin (troponin-I), or by binding calcium in the troponin structure by inhibiting actomyosin ATPase activity (O'Brien *et al.*, 2006; Kamely *et al.*, 2019). Circulating cTnI has recently been recognized as a biomarker by the American College of Cardiology for the diagnosis of cardiomyocyte damage and acute coronary syndromes (O'Brien, 2008; Kusumoto *et al.*, 2012; Sato *et al.*, 2012; Kamely *et al.*, 2019). The amount of cardiac troponin in all organisms depends on the type, duration, and degree of myocyte injury (Alpert *et al.*, 2000). Studies have reported that acute right heart failure due to myocardial damage significantly increases serum troponin T levels in broilers (Maxwell *et al.*, 1995).

One of the prognostic markers of cardiovascular disease is the red cell distribution width (RDW). The effect of RDW on mortality is frequently evaluated, and it has been shown that increased RDW level is an independent and strong risk factor for mortality in cardiovascular diseases (Salvagno *et al.*, 2015; Muhlestein *et al.*, 2016). RDW increase is associated with chronic inflammation and increased oxidative stress (Ferrucci *et al.*, 2005).

Anaemia, on the other hand, is an important precursor of cardiovascular disease. One of the indicators of anaemia is the erythrocyte index (Metivier *et al.*, 2000). These enzymes are considered reliable indicators of heat stress damage in chicken myocardial cells (Saravanan *et al.*, 2013; Tang *et al.*, 2013; Wu *et al.*, 2015). Cardiovascular problems are understood to be an important cause of sudden death in broilers (Summers, 2013). Flip-over disease (sudden death syndrome), which is seen in poultry aged 2–42 days, is a syndrome that causes severe economic losses in the broiler production industry. Antibiotics were used until 2006 to prevent these problems, but the World Health Organization subsequently stated that microorganisms gained resistance to antibiotics and could pose a risk to human health because of the animal rations under the treatment dosage (Yörük *et al.*, 2008; Çetin, 2012; Habrun *et al.*, 2012; Tekce & Mehmet, 2016). In this context, various products have been researched as feed additives to animal feeds. Research is one of the most subject of medical aromatic plants. These plants are generally characterized by secondary metabolites such as alkaloids, glycosides, and resins. These metabolites contain compounds that do not carry the risk of accumulation in animals and humans orthe ability to gain resistance to microorganisms (Burt, 2004; Feizi *et al.*, 2013). In our study, we used the essential oil mixture (EOM; Eucalyptus globulusLabill., Thymus vulgaris, Cymbopogon nardus, and Syzygiumaromaticum) used in previous studies on monogastric animals (Botsoglou *et al.*, 2004; Dezsi *et al.*, 2015), as well as anti-microbial (Daroui-Mokaddem *et al.*, 2010), anti-inflammatory (Guimarães & Quintans-Júnior, 2013), anti-viral (Silva & Pessoa, 2011), anti-fungal (Al-Ja'fari *et al.*, 2011), anti-parasitic (Kpoviessi *et al.*, 2014; Monzote *et al.*, 2007) and no side effects on blood parameters (Tekce & Gül, 2017).

This study investigated the effects ofthe above-mentioned EOM on someblood parameters and cardiac markers when added at different concentrationsto the drinking water of temperature-stressed broilers (exposed to 22°C and 36°C, respectively). With regards to the measurement of temperature stress and EOM, circulating cardiac troponin-I (cTnI) and erythrocyte indices including RDW-CV, RDW-SD MCV, MCH, MCHC, Hb, and HCT values were investigated.

MATERIALS AND METHODS

Animals, Experimental Design, Feeds

We included 400 male, one-day-old, Ross-308 chicks. The research was carried out at Bayburt University Food, Agriculture and Livestock Application and Research Centre Unit and lasted 42 days with a 35-day fattening period. During the trial, the animals were separated in 10 animals in each group with the dimensions of 110 x 110 x 100 cm for each group in the winged unit.At the end of the 7-day training period, the study groups were composed of 8 groups (22°C Control (C), C+250 mL/1,000 L, C+500 mL/1,000 L, C+750 mL/1,000 L), 36°C (stress control (SC), SC+250 mL/1,000 L, SC+500 mL/1,000 L, SC+750 mL/1,000 L) of 50 animals each with equal body weight. The treatment groups were as follows: 22°Cstress-free group (Control; EOM-250; EOM-500; EOM-750); and 36°C stress-applied volatile oil mixture(SK, SEOM-250, SEOM-500, and SEOM-750) -250, SEOM-500, and SEOM-750. Each group was divided into five subgroups

with 10 chicks each. Three different basal chick feeds and nutrient composition of the feeds are given in Table 1. This study was conducted in accordance with the ethical principles and guidelines while ensuring the protection of animal welfare and rights. The ethics committee of Atatürk University Veterinary Faculty (22.02.2018/2/24) approved this study.

Poultry House Heat Moisture and Illumination

While broiler chicks are given water ad libitum, the ration content used in their nutrition is given in Table 1. Feed and EOM were added to thewater to the working groups at the same time every day. Analysis of the feeds given to the animals was carried out according to the methods described in A.O.A.C (AOAC, 2005). During the training period, the lighting applied to the chicks was 24 hours (60 W) and the heating was 0-2. 2-5.day, at 27-28 °C temperature.

Contents of the EOM Mixture

During the study, broiler EOM mixture was added to the drinking water provided from a commercial company (Ankara, Turkey). The mixture was analyzed by GC (Gas Chromatography) in Bayburt University Central Research Laboratories. The content of the essential oil mixture was 26,70% Durenol, 23,89% Eugenol, 16,49% Gamma terpinene, 8,35% Hieptaethylene glycol, 6,42% Hexaethylene glycol, 3,31% Cymene, 3,08% Pentaethylene glycol, 2,87% Caryophyllene, 2,30% D-Limonene, 2,18% Betapinene and 0,95% Eucalyptol.

Table 1 – Basal diet ration nutrient content and analyzes (g/kg).

Raw Material	Starter (0–14 d)	Grower (14–28 d)	Finisher (28–42 d)
Maize	52.70	54.60	58.12
Maize gluten feed	15.21	21.20	26.14
Soybean residue	26.35	18.90	10.65
Di-calcium phosphate	1.95	1.70	1.60
Calcium carbonate	1.18	1.10	1.04
Sodium chloride	0.31	0.31	0.31
Sodium bicarbonate	0.20	0.20	0.20
Salt	0.2	0.2	0.2
Methionine	0.50	0.50	0.44
Lysine	1.20	1.10	1.10
Vitamin–mineral premix[1]	0.20	0.20	0.20
ME (kcal/ kg)	3100	3150	3225
Crude protein (%)	24	22	20
Crude oil (%)	2.61	2.30	2.50
Ash (%)	5.19	4.63	3.85
Moisture (%)	13.20	13.20	13.20

Vitamin-mineral premix içeriği (per kg of diet): vitamin A, 12 000 IU; vitamin D3, 1500 IU; vitamin E, 35 mg/kg; vitamin K3, 5 mg/kg; vitamin B1, 3 mg; vitamin B2, 4 mg; vitamin B6, 4 mg; vitamin B12, 0.03 mg; calcium –D-pantothenate, 15 mg; folic acit, 1 mg; niacin, 25 mg; D-biotin, 0.115 mg; Mg 80 mg/kg; I, 0,15 mg/kg; Co, 0.2 mg/kg; Cu, 5 mg/kg; Fe, 60 mg/kg; Se, 1 mg/kg; Zn, 60 mg/kg.

Collection of Serum Samples

At the end of the trial, 10 animals randomly selected from each group and 80 animals in total were categorized by 10 ml blood extraction to perform biochemical blood analysis during cervical dislocation. The refrigerated blood from the animals centrifuged (NF 1200, CORE, Ankara, Turkey) for 12 minutes at+4 °C to obtain serum samples in eppendorf tubes.

Measurement of Serum Cardiac Troponin (cTnI) level

Serum cTnI (cTnI, Sunred, Product code: 201-16-0007, China) ELISA kits, which are commercially available for the measurement of cTnI levels in blood serum samples, were obtained as a result of the study. Assay (ELISA) was evaluated by reading the absorbance values at 450 nm wave length.

Statistical analysis

The measures were all normally distributed and data are expressed as means and standard errors of the mean. Univariate general linear model was used to identify the differences existing in four diet groups. Duncan multiple comparison tests were applied in order to compare differences between the means. All statistical tests were performed at 5% level of statistical significance by IBM SPSS statistics 20.0

RESULTS

The effects of varying EOM concentrations (250, 500, and 750 mL/ 1000 L)added to the broilers' drinking water and fed under conditions of different temperature stress (22°C and 36°C) on haematological and cTnI effects are analysed in Tables 2, 3, and 4. In the groups that are not exposed to heat stress (22°C), WBC, RBC, HGB,HCT, RDW-SD, RDW-CV and Lymphocyte decreased compared to the control group, whereas cnntn-1, MCHC, neut and IG increased compared to the control group, CK, creatine, MCV, MCH, Mono, EO and Baso, and it has no effect on CK, creatine, MCV, MCH, mono, and Baso (p<0.05). In the groups exposed to a temperature stress of 36°C, the WBC, HGB, HCT, RDW-SD, and Lymphocyte decreased compared to the the control group, increased compared to MCH, MCHC, neut and IG, but had no effect on Serum cTnI, CK, creatinine, RBC, RDW-CD CD, MONO, EO and BASO (p<0.05).

Table 2 – The effects of EOM added to the drinking water of groups fed in stress conditions on biochemical blood parameters.

	CK		RDW-SD		RDW-CV		PCT		WBC		CnTn-1	
	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C
Control	5751.6	7259.6	47.80	48.57	10.90	11.12	0.00	0.00	339.76^{a}	283.18^{a}	0.25^{b}	0.75
EOM 250 ml/l	4931.0	5008.8	46.90	40.05	10.96	9.35	0.00	0.00	8.87^{b}	67.84^{b}	0.38^{b}	0.73
EOM 500 ml/l	5397.6	6099.0	40.33	45.20	9.50	10.26	0.00	0.00	69.03^{c}	54.83^{b}	0.56^{a}	0.77
EOM 750 ml/l	5976.2	5626.2	38.63	44.00	8.86	10.03	0.00	0.00	58.01^{d}	55.90^{b}	0.43^{ab}	0.79
Source of variation (p values)												
Diet	0.53		0.02		0.06		0.00		0.00		0.00	
Temperature	0.51		0.50		0.74		0.00		0.00		0.00	
Temperature x Diet	0.82		0.06		0.15		0.00		0.03		0.00	
Main effect means diet												
Control	6505.6		48.18^{a}		11.01^{a}		0.00		311.47^{a}		0.50^{b}	
EOM 250 ml/l	4969.9		43.47^{ab}		10.16^{ab}		0.00		74.36^{b}		0.60^{a}	
EOM 500 ml/l	5748.3		42.76^{b}		9.88^{ab}		0.00		61.93^{b}		0.65^{a}	
EOM 750 ml/l	5801.2		41.31^{b}		9.45^{b}		0.00		56.96^{b}		0.61^{a}	
Temperature												
22 °C	5514.1		43.41		10.05		0.00		136.92		0.40	
36 °C	5998.4		44.45		10.19		0.00		115.43		0.71	
SEM	517.5		1.06		0.29		0.00		4.44		0.02	

a,b,c: Means with the same letters in a column are statistically equal (p>0.05). Creatine Kinase (CK), Red Cell Distribution Width-Standard Deviation (RDW-SD), Red Cell Distribution Width-Coefficent of Variation (RDW-CV), Prokalsitonin (PCT), White Blood Cell (WBC),Cardiac troponin 1 (C_nT_n-1).

Table 3 – The effects of EOM added to the drinking water of groups fed in stress conditions on biochemical blood parameters.

	RBC		HGB		HCT		KREATIN		NRBC		NEUT		LYMPH	
	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C
Control	2.88	2.63	7.83	7.32	35.90	32.47	0.00	0.00	0.00	0.00	5.32	7.34	334.68	275.78
EOM 250 ml/l	2.66	2.53	7.16	6.85	32.13	30.05	0.00	0.00	0.00	0.00	39.72	32.56	40.43	34.92
EOM 500 ml/l	2.54	2.31	7.20	6.58	30.53	27.76	0.00	0.04	0.00	0.00	30.26	29.07	38.49	25.51
EOM 750 ml/l	2.47	2.34	7.35	7.13	30.50	29.30	0.02	0.04	0.00	0.00	28.51	29.17	29.32	25.73
Source of variation (p values)														
Diet	0.00		0.01		0.00		0.10		0.00		0.00		0.00	
Temperature	0.01		0.01		0.00		0.14		0.00		0.60		0.01	
Temperature x Diet	0.83		0.66		0.63		0.44		0.00		0.67		0.02	
Main effect means diet														
Control	2.75^{a}		7.58^{a}		34.18^{a}		0.00		0.00		6.33^{b}		305.22^{a}	
EOM 250 ml/l	2.59^{ab}		7.00^{b}		31.09^{b}		0.00		0.00		36.14^{a}		37.67^{b}	
EOM 500 ml/l	2.43^{bc}		6.88^{b}		29.15^{c}		0.02		0.00		29.66^{a}		32.00^{b}	
EOM 750 ml/l	2.40^{c}		7.24^{ab}		29.90^{cb}		0.03		0.00		28.84^{a}		27.52^{b}	
Temperature														
22 °C	2.64		7.38		32.26		0.01		0.00		25.96		110.73	
36 °C	2.45		6.97		29.89		0.02		0.00		24.53		90.48	
SEM	0.04		0.09		0.45		0.01		0.00		1.90		4.71	

a,b,c: Means with the same letters in a column are statistically equal (p>0.05). Red Blood Cell (RBC), Hemoglobin (HGB), Hematokrit (HCT), Nucleated red blood cells (NRBC), Nötrofil (NEUT), lymphocytes (LYMPH).

Table 4 – The effects of EOM added to the drinking water of groups fed in stress conditions on hematologic blood parameters.

	MCV		MCH		MCHC		EO		BASO		IG		MONO	
	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C	22 °C	36 °C
Control	124.70	122.60	27.33	27.65	21.76	22.50	0.01	0.29	0.10	0.20	0.19	0.14	0.22	0.12
EOM 250 ml/l	120.80	118.55	26.90	27.0	22.30	22.80	0.26	0.02	0.17	0.04	4.52	3.02	0.29	0.31
EOM 500 ml/l	120.03	119.30	28.30	29.06	23.63	24.36	0.00	0.00	0.07	0.04	3.18	2.09	0.20	0.39
EOM 750 ml/l	122.43	125.03	29.48	30.33	24.12	24.20	0.00	0.13	0.07	0.20	3.36	3.83	0.17	0.18
Source of variation (*p* values)														
Diet	0.02		0.02		0.00		0.67		0.15		0.01		0.35	
Temperature	0.59		0.43		0.20		0.67		0.61		0.48		0.71	
Temperature x Diet	0.42		0.97		0.91		0.31		0.03		0.79		0.50	
Main effect means diet														
Control	123.65^{a}		27.49^{b}		22.13^{b}		0.15		0.16^{a}		0.16^{b}		0.17	
EOM 250 ml/l	119.67^{b}		26.95^{b}		22.55b		0.14		0.12^{ab}		3.77^{a}		0.30	
EOM 500 ml/l	119.66^{b}		28.68^{ab}		24.0^{a}		0.00		0.05^{b}		2.63^{a}		0.29	
EOM 750 ml/l	123.73^{a}		29.90^{a}		24.16^{a}		0.10		0.14^{ab}		3.60^{a}		0.17	
Temperature														
22 °C	121.99		28.0		22.96		0.67		0.10		2.81		0.22	
36 °C	121.37		28.51		23.48		0.11		0.12		2.27		0.24	
SEM	0.80		0.45		0.27		0.07		0.02		0.54		0.04	

a,b,c: Means with the same letters in a column are statistically equal (p>0.05). Mean Cell Volume(MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), eosinophils (EO), immunoglobulin (IG).

DISCUSSION

With oxidative stress and increased activity of Reactive oxygen species (ROS), contractile proteins under gochemical oxidation, resulting in changes in their structural conformation or functional activity (Steinberg, 2013). Cardiac troponin I (cTnI) is a specific protein that binds to filaments in the cardiomyocyte sarcoma and affects the contraction of the heart muscle via calcium metabolism. cTnI only increases the amount of blood due to damaged myocardial tissue and cells and is normally not in the blood of healthy individuals. For this reason, in recent years, such as myocardial infarction, heart origin diseases have gained important diagnosis (Maxwell *et al.*, 1997; Fridén *et al.*, 2017). In our study, the effects of different concentrations of EOM added to broilers' drinking water fed in an environment of heat stress on cTnI were investigated on. Studies have shown that cardiac troponin T (cTnT) increases as a result of occlusive stress compared to healthy birds (Aksit *et al.*, 2008). Cardiac troponin T was an indicator of early cardiac damage before clinical manifestations. In another study, a higher value of troponin-T was determined as a result of stress caused by temperature differences (Maxwell, 1994; Maxwell *et al.*, 1995; Maxwell *et al.*, 1997; Aksit *et al.*, 2008). A similar study on broilers showed that heat stress had a negative effect on specific myofibrillar fragments, which affected the level of troponin (Santos *et al.*, 2008), heat stress applied to broiler stress and heart values have been determined to increase (Tekce & Gül, 2017). In our study, levels of cTnI were higherin the EOM-treated groups than in the control group, in groups not exposed to temperature stress (22°C). However, in the groups exposed to temperature stress (36°C), there was no effect on cTnI (p<0.05). Our results seem consistent with those in published literature.

Blood parameters vary depending on the health, environmental conditions, and dietary preferences (Gümüş & Imik, 2016). In addition to these factors, the additives that are involved in the feed and the blood values of the organism vary depending on the oxidative stress. Depending on the oxidative stress, analysis of blood samples taken on the 42nd day showed an increase in haematological values, and cultivation in high-stress environmental conditions had a marked negative effect on the blood parameters (Nezhad & Shahryar, 2011; Mahmoud *et al.*, 2011; 2013). However, in another similar study where the essential oil was added to the broiler ration under temperature stress showed no effect on haematological values (Demir *et al.*, 2008; Toghyani *et al.*, 2010; Hong *et al.*, 2012; Tekce & Rose, 2017). In the present study, we found that there was no significant difference between the groups WBC, RBC, HGB, HCT, RDW-SD, RDW-CV and Lymphocyte compared to the groups WBC, nut and IG, and it had no effect on CK, creatine, MCV, MCH, mono, EO and Baso in the groups that were not subjected to temperature stress (22 ^{O}C). In groups

exposed to temperature stress (36 OC), WBC, HGB, HCT, RDW-SD and LMYPH decreased compared to the control group, increased compared to MCH, MCHC, neut and IG, but had no effect on CK, creatine, RBC, RDW-CD, mono, EO and Baso (p<0.05). While some of the studies that we have been in compliance within the literature (Demir *et al.*, 2008; Toghyani *et al.*, 2010; Hong *et al.*, 2012; Tekce & Gül, 2017), some literature information are incompatible with the data (Nezhad & Shahryar, 2011; Mahmoud *et al.*, 2013). Discrepancies in study results are likely attributed to the manner of joining broiler rations, the dosages, and the difference in the contents of the EOM.

In conclusion, the EOM mixture did not have any effect on cardiac troponin I(cTnI) and haematocrit values. Further studies are required to better understand the addition of EOM to broiler drinking water.

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